SOME PROPERTIES OF AN AGGLUTININ IN THE HAEMOLYMPH OF THE POND SNAIL *LYMNAEA STAGNALIS*

W. P. W. VAN DER KNAAP, A. DODERER, L. H. BOERRIGTER-BARENDSEN, AND T. SMINIA*

*Biological Laboratory, Vrije Universiteit, De Boelelaan 1087, 1007 MC Amsterdam, The Netherlands, and *Department of Histology, Medical Faculty, Vrije Universiteit, P. O. Box 7161, 1007 MC Amsterdam, The Netherlands.*

ABSTRACT

A broad spectrum agglutinin in the haemolymph of the pond snail, *Lymnaea stagnalis*, is described. At dilutions ranging up to 1/2048 it agglutinated erythrocytes of several sources, bacteria and yeast cells. Haemolymph of some specimens agglutinated all types of cells, whereas that of others did not agglutinate human and sheep erythrocytes.

In inhibition tests with a variety of carbohydrates, D-galactose, L-forms of some monosaccharides, and 3 polysaccharides, especially *L. stagnalis* galactogen, were good inhibitors.

The agglutinin is a protein with a M.W. of approximately 60,000.

It was demonstrated *in vitro* that the agglutinin probably has opsonizing properties.

INTRODUCTION

Agglutinins occur in the haemolymph of gastropod molluscs and of other invertebrates (*e.g.* Pauley *et al.*, 1971; Acton and Weinheimer, 1975; Tripp, 1975; Stein and Basch, 1979). Such haemolymph agglutinins may play a role in internal defense as opsonins (Renwrantz *et al.*, 1981).

In *Lymnaea stagnalis* the haemolymph has opsonic activity (Sminia *et al.*, 1979). Also, since clotting of foreign particles was observed in the circulation of *L. stagnalis* (Van der Knaap *et al.*, 1981a), we investigated the haemolymph for the presence of agglutinins. These were found and some of their properties are described here.

MATERIALS AND METHODS

Agglutinin assays

Serum was prepared from haemolymph (Van der Knaap *et al.*, 1981a), collected from laboratory bred mature specimens (shell height 27–32 mm) of the pond snail, *Lymnaea stagnalis*. Titrations were performed in Cooke microtiter plates with V-bottomed wells of 200 μl capacity. Serum was serially two-fold diluted with phosphate-buffered saline (PBS; 0.01 M Phosphate, 0.15 M NaCl, pH 7.4) in 100 μl quantities. To each well, 100 μl of a 1% (v/v) suspension of foreign cells in PBS was added. For undiluted serum, the osmolality was adjusted to that of human serum, and 5 μl of a 10% cell suspension was added. After incubation for 2 h at room temperature titers were read macroscopically as the reciprocals of the greatest

dilutions giving total agglutination. Foreign cells used were human (bloodgroups A1, A2, B, AB and O), sheep and rabbit red blood cells, *Staphylococcus saprophyticus* and *Escherichia coli* (bacteria), and *Saccharomyces cerevisiae* (yeast). The erythrocytes were provided by the Department of Haematology, University Hospital, Vrije Universiteit, Amsterdam; the bacteria were grown overnight in Oxoid nutrient broth, harvested and washed in PBS; the yeast was purchased from a local bakery.

Adsorption tests

After the above titrations, 150 μl of supernatant fluid was carefully transferred from each well into a new well and 10 μl of a 10% cell suspension was added to each well; these cells were either of the same type as in the original test, or of another type.

Induction of agglutinin

Since agglutinin titers varied considerably among individual snails (see results), 3 experiments were performed to ascertain whether very high titers (in type I snails, see results) had been induced by previous or persistent contact between the defense system and foreign materials. In the first experiment, agglutinin titers were determined in haemolymph samples of 50 field collected specimens of *L. stagnalis*. In the second experiment, groups of 10 snails with very low agglutinin titers were injected (Van der Knaap *et al.*, 1981a) 3 times at 1 wk intervals with the following substances: 10 μl of snail Ringer (Van der Knaap *et al.*, 1981a; control snails), 10 μl of total haemolymph from snails with very high agglutinin titers, or 10 μl of snail Ringer containing 1×10^8 live bacteria (grown as described above). Bacteria used were *E. coli, S. saprophyticus*, and 4 unidentified species. The latter were isolated by spreading 100 μl quantities of snail haemolymph on nutrient broth (Oxoid) agar plates, which were then incubated at 37°C. Of the colonies which had grown overnight, 4 were selected which could be distinguished by their shape and color, and which arose from haemolymph of snails with very high agglutinin titers only. One wk after the third injection, haemolymph was sampled and agglutinin titers of individual samples were determined. It was established whether titers in experimental animals differed from those in controls. In the third experiment, 7 snails with very high agglutinin titers were kept isolated for 2 wks; then, freshly laid egg masses were collected and pooled. When the shell height of the snails hatched from these egg masses reached 27 mm, agglutinin titers were determined in haemolymph samples of 200 randomly chosen specimens.

Agglutination inhibition

In inhibition tests (and all other tests to be mentioned hereafter) only human A erythrocytes were used as foreign cells. A group of saccharides (see Table II) based on comparable published results (*e.g.* Khalap *et al.*, 1970), was tested for their capacity to inhibit agglutination. Of monosaccharides, both D- and L-isomers were tested. Saccharides were purchased from Sigma or Merck; galactogen, prepared from *L. stagnalis* albumen glands, was a gift from Prof. Dr. J. Joosse. Qualitative tests were performed by making dilution series of snail serum in PBS (see above) and adding 50 μl of PBS containing 3.6 mg of a saccharide to each well. After incubation for 1 h, 50 μl of a 2% erythrocyte suspension was added to each well, and titers were read 2 h later. Quantitative tests were performed by

making a two-fold dilution series of each saccharide (resulting in 50 μl quantities of PBS containing 7.2, 3.6, 1.8, 0.9 and 0.45 mg sugar per well) and adding 100 μl of a fixed concentration of snail serum to each well; this serum concentration contained 4 times the minimum concentration required for total agglutination in the absence of an inhibitor. After incubation for 1 h, and adding 50 μl of a 2% erythrocyte suspension, titers were read as above.

Nature of agglutinin

To test for stability of agglutinin to temperature, batches of pooled serum (from 10 snails) were heated to 40, 60, 80 and 100°C for 30 min. Also, pooled serum was stored at room temperature for 24 and 48 h. Sediments, if formed during treatment, were removed by centrifugation, and agglutination titers of the supernatant fluids were determined.

To determine the molecular nature of the agglutinin, ammonium sulphate, trichloroacetic acid (TCA), or ethanol were added to aliquots of pooled serum to final concentrations of 0, 10, 20, 30, 40 and 50% (w/v, w/v, v/v, respectively). Precipitates were removed by centrifugation and the supernatant fluids were dialyzed against 0.9% NaCl (lyophilized and dissolved in the original volume of distilled water in the case of ethanol precipitation) and agglutinin titers were determined.

Enzymatic digestion was done with trypsin (Worthington), pronase E (Merck), Lipase (Sigma), β-glucosidase (BDH), and α-amylase (Boehringer), dissolved in PBS and added to serum in final concentrations of 0.2%. To control serum, heat-inactivated enzymes or PBS were added. After incubation at 37°C for 1 h, agglutinin titrations were performed at 4°C.

Isolation of agglutinin

Since the oxygen-binding blood pigment haemocyanin binds to foreign materials (Van der Knaap *et al.*, 1981b) and might be an agglutinin, it was removed from serum (pH adjusted to 7.2) by ultracentrifugation for 1 h at $100,000 \times g$. The haemocyanin pellet was resuspended in the original volume of PBS. Agglutinin titers were determined in the supernatant fluid, in the haemocyanin suspension, and in the original serum.

Based on the results of the above mentioned experiment, additional experiments were performed to isolate agglutinin from haemocyanin-free snail serum by column chromatography. Gel filtration (Ishiyama *et al.*, 1973) on Sephadex G200 (Pharmacia), anion exchange chromatography on Cellex D (Bio Rad) and Bio-Gel A (Bio Rad), and cation exchange chromatography on SP-Sephadex C50 (Pharmacia) were tried. None of the eluted fractions had agglutinating capacity, probably because the agglutinin bound very strongly to the gel matrices (all carbohydrates). Therefore, we chose to perform gel filtration on a non-carbohydrate gel material. Of haemocyanin-free snail serum, 2 ml was eluted from a 90×0.9 cm Bio Gel P60 (polyacrylamide, Bio Rad) column with 50 mM PBS (flowrate of 1.5 ml/h). Protein-containing peaks (as determined by UV absorption) were lyophilized, dissolved in $^1/_3$ of the original volume of distilled water, and tested for agglutinating activity.

Function of agglutinin

To assess whether agglutinin has opsonizing properties, *in vitro* phagocytosis of formalized human erythrocytes by amoebocytes was measured in haemolymph

of snails with high agglutinin titers (see results, *agglutinin assays*) and in that of snails with low titers (method: Sminia *et al.*, 1979).

Results

Agglutinin assays

Agglutinating activity was detected in the haemolymph of all *L. stagnalis* specimens tested. Among individual snails titers varied considerably (0–2048, see Table I). Variation occurred according to a fixed pattern and the snails could be divided into 2 groups: those whose serum agglutinated all types of cells tested (type I snails), and those whose serum did not agglutinate human and sheep erythrocytes, but did agglutinate rabbit erythrocytes, bacteria and yeast (type II snails). Of 200 laboratory-bred snails, 184 were of type II; the remaining 16 were of type I.

Adsorption tests

During the usual titer tests the agglutinating activity of sera was reduced, presumably by adsorption to the foreign cells (final concentration 0.5% v/v). After tests with type I serum agglutination occurred in the supernatant fluids of only the first and very slightly the second well if the same type of cell was used as in the first test. If cells of another type than in the first test were used in the second test, the number of wells still containing agglutinating activity was higher; agglutinin could still be detected in the supernatant fluids of wells 1 and 2 if the titer was very high in the first test (*e.g.* 2048 with rabbit erythrocytes), and of wells 1 through 4 if the titer was low in the first test (*e.g.* 32 with *S. saprophyticus* bacteria). Of far less influence than the titer in the first test was the extent of similarity between the type of cell used in the first and the second test (*e.g.* after testing with human A1 erythrocytes, the supernatant fluid of well 1 agglutinated human A1 or A2 erythrocytes, the fluids of wells 1 and 2 agglutinated human O erythrocytes, whereas those of wells 1, 2 and 3 agglutinated *E. coli* bacteria).

After tests with type II serum, comparable results were found as with type I serum. Although human and sheep erythrocytes were not agglutinated in the first tests (see Table I), they reduced agglutinating activity of type II serum (this could

Table 1

Agglutinin titers in L. Stagnalis *serum.*

Foreign cell tested	Titer in type 1 snails	Titer in type II snails
human A1 erythrocytes	256–1024	0
human A2 erythrocytes	128–512	0
human B erythrocytes	128–512	0
human AB erythrocytes	128–512	0
human O erythrocytes	256–1024	0
sheep erythrocytes	128–512	0
rabbit erythrocytes	1024–2048	4–8
S. saprophyticus	16–64	8–32
E. coli	256–1024	256–1024
Sacch. cervisiae	256–1024	256–1024

Highest and lowest titers (reciprocals of the greatest dilutions giving total agglutination) found for 16 type I snails and 184 type II snails are given.

be measured with rabbit erythrocytes, the bacteria, and yeast cells only, see Table I) to the same extent as they did in type I serum.

Induction of agglutinin

Of 50 field collected snails, 49 were type II and 1 was type I. In the haemolymph of all 50 specimens unidentified parasites and bacteria were observed with phase-contrast microscopy. It therefore is unlikely that the higher agglutinin titers in type I snails were induced non-specifically by persistent contact between the defense system and these foreign organisms.

Agglutinin titers in type II snails were unaltered after repeated injections with any of 6 bacterial species (4 of which had been isolated from type I snails), or with type I haemolymph. This makes it highly improbable that certain bacteria or viruses would have specifically induced higher agglutinin titers in type I snails.

Of 200 specimens reared from egg masses laid by type I snails, 120 were type I, whereas in the normal laboratory snail stock only 16 out of 200 were type I. Therefore, it is likely that heredity rather than induction determines whether a snail is of type I.

Agglutination inhibition

Agglutination of human A erythrocytes in type I serum was inhibited by carbohydrates. Since the inhibitory effect of each saccharide varied per experiment, ranges of effectivity observed in 4 qualitative and 4 quantitative tests are listed in Table II.

Nature of agglutinin

Type I serum, heated to 40°C for 30 min, or stored at room temperature for 24 h agglutinated human A erythrocytes with the same titers as fresh serum. If

TABLE II

Inhibition of L. stagnalis *agglutinin by saccharides.*

Monosaccharides	D-Isomer	L-Isomer	Other saccharides	
arabinose	±	±	lactose	±
fructose	0	N.D.	trehalose	±
fucose	0	+	galactosamine	0
galactose	+	+	N-ac-galactosamine	±
glucose	±	++	N-ac-glucosamine	±
mannose	±	++	cellulose	+
rhamnose	N.D.	+	galactogen	+++
ribose	0	N.D.	glycogen	++
xylose	±	±	starch	0

Pooled results of 4 qualitative and 4 quantitative experiments. The range of the inhibitory effects which each sugar (final concentration 18 mg/ml) had in the 4 qualitative experiments is expressed as: 0, inhibition was not observed; ±, titers not reduced–reduced 2 fold; +, titers reduced 2–4 fold; ++, titers reduced 4–8 fold; +++, titers reduced 8–16 fold (*e.g.*, if in the 4 experiments titers without addition of an inhibitor were 512, 512, 256 and 512, and after addition of an inhibitor titers were 256, 128, 128 and 256 respectively, this sugar reduced titers 2, 4, 2 and 2 fold, and is therefore listed as + for 2–4 fold reduction in titer). The same signs are used to indicate how the inhibiting effects of the sugars ranged in 4 quantitative experiments: 0, inhibition was not observed; ±, no inhibition–inhibition with 36 or 18 mg/ml sugar concentration; +, inhibition with 9–18 mg/ml sugar concentration; ++, ditto, 4.5–9 mg/ml; +++, ditto, 2.25–4.5 mg/ml (*e.g.*, if a sugar worked inhibitory at concentrations 18, 9, 9 and 18 mg/ml in the 4 experiments, it is listed as +. N.D.: not done.

Agglutinin titers of serum after addition of ammonium sulphate (in % w/v final concentration), TCA (% w/v), or ethanol (% v/v).

Precipitant	% final concentration					
	0	10	20	30	40	50
Amm. sulphate	1024	1024	1024	512	512	0
TCA	1024	0	0	0	0	0
Ethanol	1024	1024	512	2	0	0

stored at room temperature for 48 h, titers decreased from 1024 to 64. Serum heated to 60, 80 or 100°C for 30 min lost agglutinating activity completely.

The addition of ammonium sulphate, TCA or ethanol to type I serum resulted in a marked precipitation of material, and supernatant fluids lost agglutinating capacity (Table III).

The above results indicate that the agglutinin is proteinaceous. This is corroborated by enzymatic digestion. Control sera (incubated with PBS or heat-inactivated enzymes) agglutinated with titers of 512, whereas sera treated with trypsin or pronase had no detectable agglutinating activity. Lipase, β-glucosidase and α-amylase did not affect agglutinin titers.

Isolation of agglutinin

The agglutinin titer of normal haemolymph was 1024; after removal of haemocyanin the supernatant fluid had a titer of 512 and the resuspended haemocyanin had a titer of 4.

With gel filtration on Bio-Gel P60 most of the agglutinating activity was found in a conspicuous shoulder at the end of the void volume; a small peak eluted later also contained some agglutinating activity. Two other peaks of lower molecular weight materials, but without agglutinating activity, were eluted.

Function of agglutinin

The opsonizing activity of haemolymph (Sminia *et al.*, 1979) is dependent on its agglutinating activity: both the percentage of phagocytosing amoebocytes and the number of ingested foreign cells per amoebocyte reached maximum values within 30 min in type I haemolymph, whereas in type II haemolymph a 45 min exposure was required before these maxima were reached (see Fig. 1).

DISCUSSION

In the present study the suggestion (Van der Knaap *et al.*, 1981a) that heamolymph of *L. stagnalis* would contain agglutinin was confirmed. With human A erythrocytes, serum of a minority of snails (type I) appeared to have very high titers, whereas serum of the majority of snails (type II) seemed to lack agglutinin. After testing a panel of foreign cells it became evident that type II snails do not lack agglutinin, but have a substance which agglutinates only micro-organisms and rabbit red blood cells. Although this substance does not agglutinate human or sheep erythrocytes, the adsorption tests showed that it does bind to these foreign cells. This is a property which it has in common with type I agglutinin. Possibly type I and type II agglutinin are modifications of one molecule, the latter lacking one
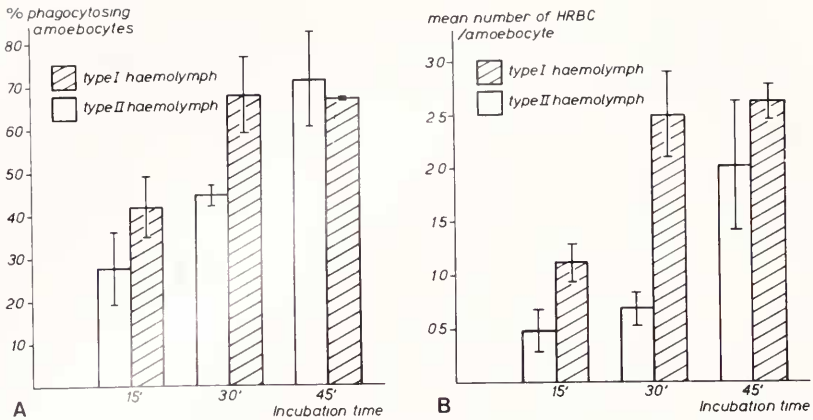
FIGURE 1. *In vitro* phagocytic activity of amoebocytes in pooled haemolymph of 5 type I snails (dashed columns) and of 5 type II snails (open columns). Phagocytosis was stopped after 15, 30 and 45 min, and the percentages of amoebocytes (n = 300) that had phagocytosed (Fig. 1A) and the mean numbers of ingested human erythrocytes per amoebocyte (Fig. 1B) were determined. Means ± S.D. of 3 parallel experiments are given.

or more receptor sites (so that sufficient cross-linking between erythrocytes can not occur). This supposition is in agreement with the observation that, besides hae-mocyanin, only one substance in pooled snail haemolymph binds to foreign cells (Van der Knaap *et al.*, 1981b).

Differences in agglutinin titers among haemolymph samples of different species within one molluscan genus (Gilbertson and Etges, 1967; Michelson and Dubois, 1977), of different strains within one species (Gilbertson and Etges, 1967; Stan-islawski *et al.*, 1976; Michelson and Dubois, 1977), and of different specimens within one strain (Michelson and Dubois, 1977) appear to be a general phenomenon. It has been suggested that it could be of use for taxonomic purposes (Gilbertson and Etges, 1967; Michelson and Dubois, 1977). Repeated injections of type II snails with type I total haemolymph, or with bacteria which could be isolated from type I snails only, did not result in higher titers. Therefore it is unlikely that the stronger agglutinin in type I snails was induced by a viral or bacterial infection. A snail's agglutinin type (I or II) is apparently genetically determined, since 60% of the progeny of type I snails that had been isolated for several weeks were of type I, whereas in the normal laboratory stock this percentage was 8.

In the present study rabbit erythrocytes were the red cells that were agglutinated best. Some other molluscan sera show a similar activity in this respect (*e.g.* Tripp, 1966; Gilbertson and Etges, 1967; Jenkin and Rowley, 1970). Sera of 6 specimens of *Viviparus malleatus* agglutinated rabbit red cells only, and gave titers compa-rable to those found in type II haemolymph in the present study (Cheng and Sanders, 1962). Possibly in this species differences in agglutinating activity com-parable to those in *L. stagnalis* occur among individual snails.

The agglutinins in *L. stagnalis* work non-specifically, since they have a broad action spectrum: type II serum agglutinates at least 3 species of micro-organisms plus rabbit red cells, whereas type I agglutinin is in addition directed against sheep red cells and human erythrocytes, without showing ABO bloodgroup specificity. Another argument for the non-specificity of *L. stagnalis* agglutinin is that adsorp-tion of serum with any of the foreign cells markedly reduced titers against all other

cell types. In addition, the non-specificity of *L. stagnalis* agglutinin was evidenced by the inhibition tests: of 24 sugars tested, 9 inhibited the agglutinin in all experiments.

Of 8 D-isomers of monosaccharides, only D-galactose was a good inhibitor: although giving only slight inhibition, it was effective in all experiments. Interaction between D-galactose and molluscan agglutinins is not unusual (Khalap *et al.*, 1970; Baldo *et al.*, 1977). On the other hand, of 7 L-isomers, 5 were good inhibitors. It is tempting to state that these biologically uncommon sugars would be receptor sites par excellence for recognition factors. Strikingly, N-ac-galactosamine and related sugars, which are strong inhibitors of molluscan agglutinins (*e.g.* McDade and Tripp, 1967; Kühnemund and Köhler, 1969; Khalap *et al.*, 1970; Arimoto and Tripp, 1977; Michelson and Dubois, 1977; Uhlenbruck *et al.*, 1979) had little or no effect on *L. stagnalis* agglutinin. Polysaccharides seem to be stronger inhibitors than the monomers that they are composed of: cellulose and glycogen were good inhibitors, whereas D-glucose was not; obviously the nature of the linkage between the monosaccharides is essential, since starch had no inhibiting effects. Poly-D-galactose, *i.e. L. stagnalis* galactogen, proved to be by far the best inhibitor in the present study. This is not surprising, since galactogens interact strongly with molluscan agglutinins (Uhlenbruck *et al.*, 1979).

The agglutinin is obviously of proteinaceous nature because it was precipitated by ammonium sulphate, ethanol, and TCA; it was unstable to heat and was inactivated by pronase and by trypsin. Since it was not affected by enzymes which degrade lipids and carbohydrates the molecule may be pure protein, or it may contain carbohydrate and lipid moieties which are either masked or not essential for the functioning of the molecule. The agglutinin is not identical with the blood pigment haemocyanin. This was corroborated by the results of gel filtration on Bio-Gel P60 columns. Since protein with agglutinating power was eluted in a shoulder of the void volume, the M.W. must be just less than 60,000. The small peak in the lower M.W. range with agglutinating activity may have contained agglutinin subunits.

Molluscan agglutinins have been shown to act as opsonins, both *in vitro* (*e.g.* Arimoto and Tripp, 1977) and *in vivo* (*e.g.* Renwrantz *et al.*, 1981). Opsonizing activity was demonstrated in the serum of *L. stagnalis* by Sminia *et al.* (1979), and indications were found (Van der Knaap *et al.*, 1981b) that this opsonin is identical with the agglutinin described in the present study. The present study is in agreement with the concept that *L. stagnalis* agglutinin has opsonizing properties: in *in vitro* phagocytosis both the percentage of amoebocytes phagocytosing human erythrocytes, and the number of red cells engulfed per amoebocyte were higher in the stronger agglutinating type I, than in type II haemolymph.

LITERATURE CITED

ACTON, R. T., AND P. F. WEINHEIMER. 1975. Hemagglutinins: Primitive receptor molecules operative in invertebrate defense mechanisms. Pp 271–282 in E. L. Cooper, Ed., *Contemporary Topics in Immunobiology* Vol. **4**. Plenum Press, New York.

ARIMOTO, R., AND M. R. TRIPP. 1977. Characterization of a bacterial agglutinin in the hemolymph of the hard clam, *Mercenaria mercenaria. J. Invertebr. Pathol.* **30**: 406–413.

BALDO, B. A., G. UHLENBRUCK, AND G. STEINHAUSEN. 1977. Invertebrate anti-galactans. A comparative study of agglutinins from the clam *Tridacna maxima*, the marine sponge *Axinella polypoides* and the anemone *Cerianthus membranaceus. Comp. Biochem. Physiol.* **56A**: 343–351.

CHENG, T. C., AND B. G. SANDERS. 1962. Internal defense mechanisms in molluscs and an electrophoretic analysis of a naturally occurring serum hemagglutinin in *Viviparus malleatus* Reeve. *Proc. Pa. Acad. Sci.* **36**: 72–83.

GILBERTSON, D. E., AND F. J. ETGES. 1967. Haemagglutinins in the haemolymph of planorbid snails. *An. Trop. Med. Parasitol.* **61**: 144–147.

ISHIYAMA, I., W. DIETZ, AND G. UHLENBRUCK. 1973. Comparative study of anti-A-agglutinins from various snails of the genus *Helix (Helix pomatia* and *Helix aspersa). Comp. Biochem. Physiol.* **44B**: 529–547.

JENKIN, C. R., AND D. ROWLEY. 1970. Immunity in invertebrates. The purification of a haemagglutinin to rat and rabbit erythrocytes from the haemolymph of the murray mussel *(Valesunio ambiguus). Aust. J. Exp. Biol. Med. Sci.* **48**: 129–137.

KHALAP, S., T. E. THOMPSON, AND E. R. GOLD. 1970. Haemagglutination and haemagglutination-inhibition reactions of extracts from snails and sponges. I. Agglutination of human and various animal red cells: its inhibition by sugars and aminosugars. *Vox Sang.* **18**: 501–526.

KÜHNEMUND, O., AND W. KÖHLER. 1969. Untersuchungen über die Reinigung des Protektins Anti-A_{hel} (Anti-A_{HP}) aus *Helix pomatia. Experientia* **25**: 1137–1138.

MCDADE, J. E., AND M. R. TRIPP. 1967. Mechanisms of agglutination of red blood cells by oyster hemolymph. *J. Invertebr. Pathol.* **9**: 523–530.

MICHELSON, E. H., AND L. DUBOIS. 1977. Agglutinins and lysins in the molluscan family Planorbidae: A survey of hemolymph, eggmasses and albumen-gland extracts. *Biol. Bull.* **153**: 219–227.

PAULEY, G. B., G. A. GRANGER, AND S. M. KRASSNER. 1971. Characterization of a natural agglutinin present in the hemolymph of the California sea hare, *Aplysia californica. J. Invertebr. Pathol.* **18**: 207–218.

RENWRANTZ, L., W. SCHÄNKE, H. HARM, H. ERL, H. LIEBSCH, AND J. GERCKEN. 1981. Discriminative ability and function of the immunobiological recognition system of the snail *Helix pomatia. J. Comp. Physiol.* **141**: 477–488.

SMINIA, T., W. P. W. VAN DER KNAAP, AND P. EDELENBOSCH. 1979. The role of serum factors in phagocytosis of foreign particles by blood cells of the freshwater snail *Lymnaea stagnalis. Dev. Comp. Immunol.* **3**: 37–44.

STANISLAWSKY, E., L. RENWRANTZ, AND W. BECKER. 1976. Soluble blood group reactive substances in the hemolymph of *Biomphalaria glabrata* (Mollusca). *J. Invertebr. Pathol.* **28**: 301–308.

STEIN, P. C., AND P. F. BASCH. 1979. Purification and binding properties of hemagglutinin from *Biomphalaria glabrata. J. Invertebr. Pathol.* **33**: 10–18.

TRIPP, M. R. 1966. Hemagglutinin in the blood of the oyster *Crassostrea virginica. J. Invertebr. Pathol.* **8**: 478–484.

TRIPP, M. R. 1975. Humoral factors and molluscan immunity. Pp. 201–223 in K. Maramorosch and R. E. Shope, Eds., *Invertebrate Immunity.* Academic Press, New York, San Francisco, London.

UHLENBRUCK, G., D. KARDUCK, AND R. PEARSON. 1979. Different tridacnins in different tridacnid clams: a comparative study. *Comp. Biochem. Physiol.* **63B**: 125–129.

VAN DER KNAAP, W. P. W., T. SMINIA, F. G. M. KROESE, AND R. DIKKEBOOM. 1981a. Elimination of bacteria from the circulation of the pond snail *Lymnaea stagnalis. Dev. Comp. Immunol.* **5**: 21–32.

VAN DER KNAAP, W. P. W., L. H. BOERRIGTER-BARENDSEN, D. S. P. VAN DEN HOEVEN, AND T. SMINIA. 1981b. Immunocytochemical demonstration of a humoral defence factor in blood cells (amoebocytes) of the pond snail, *Lymnaea stagnalis. Cell Tissue Res.* **219**: 291–296.